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Development and application of a simple routine method for the determination of selenium in serum by octopole reaction system ICPMS

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Abstract The aim of the study was to develop an inductively coupled plasma mass spectrometry (ICPMS) method for robust and simple routine determination of selenium in serum. Polyatomic interferences on ^{76}Se, ^{77}Se, and ^{78}Se were removed by applying an octopole reaction system ICPMS with the reaction cell pressurized with H_2 gas. We developed a novel simple optimization routine for the H_2 gas flow based on a signal-to-noise ratio (SNR) calculation of the selenium signal measured in a single selenium standard. The optimum H_2 flow was 2.9 mL min^{-1}. The selenium content in serum was determined after a 50-fold dilution with 0.16 M HNO_3 and quantified by using addition calibration and gallium as an internal standard. The method detection limit was 0.10 μg L^{-1} for ^{76}Se and ^{78}Se and 0.13 μg L^{-1} for ^{77}Se. Human serum samples from a case-control study investigating if selenium was associated with risk of colorectal adenoma were analyzed. The average selenium concentration for the control group ($n=768$) was 137.1 μg L^{-1} and the range was 73.4–305.5 μg L^{-1}. The within-batch repeatability (a batch is ten samples) estimated from 182 replicate analyses was 6.3% coefficient of variation (CV), whereas the between-batch repeatability was 7.4% CV estimated from 361 replicates between batches. The method accuracy was evaluated by analysis of a human serum certified reference material (Seronorm Serum level II, Sero A/S, Norway). There was a fairly good agreement between the measured average of 145 ± 3 μg L^{-1} ($n=36$) and the certified value of 136 ± 9 μg L^{-1}. In addition the method was successfully applied for analysis of zinc serum concentrations without further optimization. For the Seronorm certified reference material a value of 911 ± 75 μg L^{-1} ($n=31$) for zinc was obtained, which corresponds well to the certified zinc value of 920 ± 60 μg L^{-1}.

Keywords ICPMS · Selenium · Serum · Octopole reaction system · Signal-to-noise optimization · Interferences

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Introduction

Selenium is an essential trace element which is incorporated in the active center of antioxidant selenoenzymes (glutathione peroxidases and thioredoxin reductases). Owing to these antioxidative properties, which prevent oxidative damage to DNA and other important biomolecules, selenium may prevent cancer [1, 2]. Some observational studies as well as a randomized prevention trial showed that selenium reduced the risk for different cancers such as colorectal, lung, and prostate cancer [1, 2]. To further investigate this interesting hypothesis epidemiological studies including a large randomized clinical trial [3] are currently underway. These studies will primarily rely on selenium measurements in serum and other biological materials such as toenails, rather than dietary questionnaires to estimate selenium intake. The reasoning behind this is that the selenium concentrations of the same foods vary largely depending on the selenium content of the soil where plants are grown and hence a dietary questionnaire cannot capture such variations [4]. In epidemiological studies serum selenium levels of cancer cases are compared to those from controls without cancer, which are randomly selected from the same source population as the cases.

We present here a robust and simple routine method for serum selenium analysis that we used for a nested case-control study on the association between selenium and risk for colorectal adenoma, a well-known colorectal cancer precursor. Several case-control studies suggest that selenium may reduce the risk of colorectal cancer [5–9]. However, most of these studies are small with less than 100 cancer cases and a similar number of controls. Therefore, most studies did not find significant differences in selenium levels between cases and controls, particularly, because these studies suggest that the differences between cases and controls are rather small. However, given the broad application of selenium prevention even small effects will be important with respect to public health and could substantially contribute to reduce the burden of this severe disease [10]. Because measurement error further reduces the difference in selenium levels between cases and controls it is important to measure serum selenium levels with a precise method, which at the same time can analyze several hundred serum samples needed for sufficiently powered studies.

The determination of selenium is traditionally performed by atomic absorption spectrometry (AAS), in particular hydride generation AAS, which is a very sensitive technique [11]. During the last two decades the number of inductively coupled plasma mass spectrometry (ICPMS) instruments available in analytical laboratories has increased rapidly and several methods applying ICPMS for selenium determination in many different sample matrices have been published [12]. The determination of selenium by ICPMS is hampered by a large number of polyatomic ions interfering with all of selenium's six isotopes [13]. Recent developments in ICPMS technology like high-resolution sector field ICPMS and reaction/collision cell ICPMS provides means of elevating many of these interferences and have improved the selenium measurement capabilities of ICPMS significantly [14–19]. Human serum is a difficult matrix to analyze by ICPMS as it contains 6–8% proteins and 1% inorganic salts [20], hence both spectral and non-spectral interferences need to be considered. A number of ICPMS methods for the determination of selenium in serum which utilize different interference elevation strategies have been published. A method for determining serum selenium after an eightfold dilution with 0.14 M HNO_3 using the ^{77}Se isotope and a mathematical correction for the $^{40}Ar^{37}Cl^+$ overlap was published by Vanhoe et al. [13]. The validity of the mathematical interference correction, and thereby the accuracy of the method, rests on the assumption that the $^{40}Ar^{35}Cl^+$:$^{40}Ar^{37}Cl^+$ ratio is constant in all solutions analyzed. Selenium has a relatively high ionization potential (IP 9.75 eV) and is only partially ionized in the ICP (33% [21]); it has been shown that the addition of an organic carbon source (e.g., methanol) to the sample solutions can increase the ionization of selenium in the ICP significantly [21, 22]. This has been utilized in several analytical methods to increase the sensitivity of selenium and to ensure an even ionization efficiency in all analyzed solutions [23–26]. Gossens et al. [23] were able to determine selenium in serum by using the ^{77}Se and ^{78}Se isotopes following an eightfold dilution with nitric acid (1%) and ethanol (4%) which combined with a high nebulizer gas flow reduced the interferences from $ArCl^+$ and Ar_2^+ sufficiently to allow analysis. Quantification was performed by using a combined standard addition and internal standard (gallium) approach to eliminate non-spectral interferences (matrix effects). By using a similar approach Labat et al. [26] successfully used the ^{82}Se isotope following a tenfold dilution of the serum with a solution containing nitric acid (1%), X-triton (0.1%), and 1-butanol (0.8%). By applying a reaction/collision cell ICPMS, Nixon et al. [25] removed the Ar_2^+ dimer at m/z 78 and 80 using methane as a reaction gas in a dynamic reaction cell. Selenium was then measured with good accuracy and precision after a 20-times dilution with a diluent containing 10% v/v ethanol, 1% nitric acid, 0.5% Triton X-100, and Ga and Y as internal standards. After a tenfold dilution with 3% butanol, 0.1% TAMA (a high-purity surfactant), and 0.05% HNO_3, Nelms [24] successfully measured selenium in serum on $m/z=80$ and 82 with a reaction/collision cell ICPMS instrument. ^{80}Se was determined in the reaction mode with a mixed H_2/He reaction gas, whereas ^{82}Se was determined in the standard mode without a reaction gas. There was no significant difference in serum selenium between results obtained using the two isotopes. Reyes et al. [27] determined selenium in serum after a tenfold dilution with deionized (DI) water using isotope dilution with enriched ^{77}Se and octopole reaction/collision cell ICPMS. They were able to measure the $^{78}Se/^{77}Se$ and $^{80}Se/^{77}Se$ isotope ratios with a precision of 0.2% RSD and quantified selenium accurately in a certified serum reference material. Featherstone et al. [19] employed a different interference removal approach: by applying a high-resolution ICPMS (RP = 7,500) they were able to measure selenium in serum on $m/z=77$ and 82 after dilution with DI water, acidification with nitric acid, and addition of ethanol. In the low-resolution mode (RP = 300), only ^{82}Se gave accurate results since ^{77}Se were then interfered by $^{40}Ar^{37}Cl$.

This paper presents a simple and robust analytical method for the determination of serum selenium by employing a 50-fold dilution with 0.16 M HNO_3 and removal of Ar_2^+ and $ArCl^+$ interferences with H_2 as a reaction gas in an octopole reaction/collision cell. Selenium is measured by using ^{76}Se, ^{77}Se, and ^{78}Se and quantified with a combined standard addition and internal standard (gallium) procedure. A novel simple optimization procedure of the H_2 gas flow based on the measurement of a single aqueous selenium standard solution was developed; this allows for a accurate optimization of the H_2 flow. The developed method was subsequently applied for the analysis of 1,800 serum samples from a cancer research study with the aim of investigating the association of serum selenium with risk of colorectal adenoma, a cancer precursor. The results of this study will be published elsewhere. The quality control data from this applied study is discussed in the present paper.

Experimental

Instrumentation

All measurements were performed on an Agilent 7500c ICPMS instrument with an octopole reaction system (Agilent Technologies Inc, Palo Alto, CA, USA) in operation at the Dartmouth Trace Element Analysis Core Facility, Dartmouth College, NH, USA. The instrument was operated by using the standard setup comprised of a Scott-type spray chamber and a Babbington nebulizer. An internal standard solution (gallium) was continuously mixed with the sample flow in a 1:25 ratio using a T-connection and the built-in peristaltic pump. Throughout this study the octopole cell was pressurized with ultra high purity hydrogen gas (>99.999%) with a H_2O content below 2 ppm (Merriam-Graves Corporation, Charlestown, NH, USA). The instrument settings and the method parameters are summarized in Table 1.

Chemicals

All samples, reference material, and standards were prepared using 0.16 M HNO_3 made from ultrapure water (>18.2 M cm) produced by a Purelab Plus water purifier (US Filter, MA, USA) and concentrated 69% Optima nitric acid (Fisher Scientific, Pittsburgh, PA, USA). Dilute standard solutions of Se, Ga, Ca, and Br were made from the following single element standard solutions: 1,000 μg L^{-1} selenium (SCP Science, Champlain, NY, USA), 10 mg L^{-1} gallium and 100 mg L^{-1} bromine (High-purity standards, Charleston, SC, USA), and 1,000 mg L^{-1} calcium (VHG labs, Manchester, NH, USA).

Table 1 Acquisition parameter for the Agilent 7500c ICPMS

Power	1,600 W
Sampling depth	6 mm (optimized daily)
Carrier gas	1.2 L min^{-1}
Plasma gas	15 L min^{-1}
Auxiliary gas	0.9 L min^{-1}
Extraction voltage	4.5 V (optimized daily)
Octopole bias	−14.4 V
Quadrupole bias	−12.4 V
H_2 gas flow	2.6–3.2 mL min^{-1} (optimized daily)
Isotopes	^{71}Ga (internal standard), ^{76}Se, ^{77}Se, ^{78}Se
Dwell time	100 ms
Sensitivity	^{89}Y: 15–20,000 cps/ppb (0.16 M HNO_3) ^{78}Se: 3–500 cps/ppb (50 times diluted serum)
Points per peak	3
Scans	8
Analysis time	15 s
Uptake time	50 s
Wash time	60 s
Sample preparation	50-fold dilution with 0.16 M HNO_3

Samples

The samples are from a case-control study investigating if selenium is associated with risk of colorectal adenoma conducted by the National Cancer Institute (NCI), Rockville, Maryland, USA. The results of this study will be published elsewhere [28]. The study is part of a large cancer screening trial, the prostate, lung, colorectal, and ovarian cancer (PLCO) screening trial, to investigate the effectiveness of early detection for these cancers and to identify early markers and etiologic determinants of cancer [29, 30]. A total of about 155,000 participants were recruited at ten different screening centers (Birmingham AL, Denver CO, Detroit MI, Honolulu HI, Marshfield WI, Minneapolis MN, Pittsburgh PA, Salt Lake City UT, St Louis MO, and Washington DC). The trial recruited men and women aged 55–74 years from the general population. Approximately 1,800 frozen serum samples (300 μL) for selenium analysis were shipped on dry ice (carbon dioxide at −78°C) and stored at the Dartmouth Trace Element Analysis Core Facility at −70°C until analysis. Samples were thawed on the day of analysis, 50 μL was taken out for analysis, and the remaining sample was re-frozen. The method development and the interference studies were carried out by using a human serum certified reference material (Seronorm Serum level II, Sero A/S, Norway). The material is certified to contain 136 ± 9 μg L^{-1} selenium. This material was furthermore used as a quality control sample, and a sub-sample was analyzed with every batch of serum samples from the PLCO screening trial.

Sample preparation, measurement, and calculations

The sample preparation was kept simple, i.e., a 50-fold dilution with 0.16 M HNO_3. One day of analysis consisted of five batches of ten serum samples; however, it is possible to analyze up to 100 samples a day. We obtained for every ten serum samples a 5-point addition calibration curve measured in the serum reference material. The five sub-samples were spiked with 0, 1, 2, 4, and 6 μg L^{-1} selenium. In addition, we measured two blank solutions (0.16 M HNO_3) for every ten serum samples. An internal standard (100 μg L^{-1} gallium) was continuously added to the sample solution flow. All solutions were analyzed using the acquisition parameters outlined in Table 1. The raw data were exported to an Excel spreadsheet. All data were normalized using the internal standard. We calculated the selenium content in the serum samples by using the average slope of the five calibration curves for the five batches analyzed daily. All results were calculated as the average of the concentrations obtained for ^{76}Se, ^{77}Se, and ^{78}Se, respectively. The 1,800 serum samples were analyzed over a period of 8 months.

Results and discussion

Interferences

Non-spectral interferences

Human serum is a complex sample containing high concentrations of proteins (6–8%) and inorganic salts (1%), particularly salts with sodium and potassium at high mg L^{-1} levels [20]. When dilute serum samples are introduced into ICPMS, instrument space charge effects occur mainly in the interface region between the skimmer and the ion lenses causing the overall analyte sensitivity to drop. Space charge effects are the repulsion of like charges when a high charge density occurs in the ion beam from the ICP. In the ion beam behind the skimmer cone the positive ions are present at a high density, above the space charge limit, so they repel each other and ions (primarily lighter ions) are lost from the ion beam [31]. Also the nebulization efficiency is most likely affected by the serum sample matrix, which probably decrease the observed signal as the nebulizer gas flow rate found to be optimum for a 0.16 M nitric acid solution (and used for the analysis of serum samples) might not be the optimum nebulizer gas flow for the serum samples. As a consequence of these effects a drop in selenium sensitivity of 5–10%, estimated by the change in the slopes of the calibration curves obtained for aqueous standards versus dilute serum samples, was observed when analyzing serum diluted 50-fold with 0.16 M HNO_3. As a consequence we performed all quantifications with addition calibration by applying a 5-point standard addition calibration curve in the serum reference material. No difference was observed in sensitivity between the reconstituted serum reference material and the real serum samples.

Addition of organic-bound carbon is known to increase the sensitivity of selenium in ICPMS by facilitating an increased ionization efficiency of selenium in the ICP [21, 22]. For the determination of selenium in human serum the addition of a few percent of organic solvent (ethanol or butanol) to the sample solutions is often used to match the carbon content between individual samples and aqueous standards, thereby ensuring that the ionization efficiency of selenium is the same in all solutions [23–26]. Addition of an organic-carbon source was not found necessary in this study as calibration in a serum matrix was applied. Furthermore we did not observe a difference in selenium sensitivity between individual serum samples or between serum samples and the serum reference material, as no significant differences in the slopes of standard addition calibration curves in different samples or the serum reference material were observed. Part of the explanation for this observation is probably that the serum samples were diluted 50 times thus resulting in a relatively low carbon concentration having little influence on the ionization efficiency. In the previous published studies a dilution factor of 8–20 was used resulting in a higher final carbon content of the sample solutions and more pronounced differences in the carbon content between individual samples and between samples and standards [23–26].

Spectral interferences

One of the main difficulties in the determination of selenium with ICPMS is elimination of the many polyatomic ions that interfere with the signals of all selenium isotopes. Most severe is the interference from $^{40}Ar_2^+$ on $^{80}Se^+$, selenium's most abundant isotope. We summarized the most common interferences on the selenium isotopes in Table 2. Different approaches to solve this problem and enable accurate and precise selenium analysis in serum by ICPMS have been published (e.g., mathematical interference correction [13], ethanol addition combined with nebulizer gas flow adjustment [23], or use of a reaction/collision cell ICPMS [24, 25]). In the method presented in this paper an octopole reaction system ICPMS instrument was applied to almost completely remove the argon-based interferences upon chemical reaction with hydrogen gas (H_2). The chemical reaction is believed to follow the general equation below [15], illustrating how the $^{40}Ar_2^+$ ions are dissociated leaving $m/z = 80$ free for the selenium analysis while increasing the ArH^+ signal at $m/z = 41$.

$$^{40}Ar_2^+ + H_2 \Rightarrow ^{40}ArH^+ + ^{40}Ar + H$$

In a similar fashion $^{38}Ar^{40}Ar^+$ and $^{40}Ar^{37}Cl^+$ are also dissociated leaving $^{77}Se^+$ and $^{78}Se^+$ free for analysis. Other interferences also need to be considered: calcium, for instance, is present in serum at relatively high levels (100 mg L^{-1}) and consequently $ArCa^+$ is a potential interference on ^{76}Se, ^{78}Se, ^{80}Se, and ^{82}Se. With a H_2 gas flow of 2.6–3.5 mL min^{-1} and a 2 mg L^{-1} Ca standard

Table 2 Spectral interferences on selenium. The interferences in bold are removed with H_2 (the reaction gas) in the octopole

Isotope	Abundance (%)	Interference
^{74}Se	0.89	$\mathbf{^{36}Ar^{38}Ar^+}$, $^{37}Cl_2^+$, $\mathbf{^{42}Ca^{16}O_2^+}$, $\mathbf{^{40}Ca^{16}O^{18}O^+}$, $^{74}Ge^+$
^{76}Se	9.37	$\mathbf{^{38}Ar_2^+}$, $\mathbf{^{44}Ca^{16}O_2^+}$, $\mathbf{^{36}Ar^{40}Ca^+}$, $^{76}Ge^+$
^{77}Se	7.63	$\mathbf{^{40}Ar^{37}Cl^+}$, $^{76}SeH^+$
^{78}Se	23.77	$\mathbf{^{40}Ar^{38}Ar^+}$, $\mathbf{^{38}Ar^{40}Ca^+}$, $^{77}SeH^+$
^{80}Se	49.61	$\mathbf{^{40}Ar_2^+}$, $\mathbf{^{40}Ar^{40}Ca^+}$, $^{79}BrH^+$, $\mathbf{^{32}S^{16}O_3^+}$, $^{80}Kr^+$
^{82}Se	8.73	$\mathbf{^{40}Ar^{42}Ca^+}$, $^{81}BrH^+$, $\mathbf{^{34}S^{16}O_3^+}$, $^{82}Kr^+$

solution (equivalent to the Ca concentration in 50-fold diluted serum) we did not observe any $ArCa^+$. Therefore, we concluded that interference from calcium ions is not a problem at the calcium concentration level present in dilute serum, since either $ArCa^+$ is not formed or the H_2 reaction gas in the octopole reaction cell dissociates it.

While many interferences are removed, other interferences are formed: one of these is SeH, which makes the determination of selenium isotopes ratios difficult, but it has no effect on total determinations of selenium as the formation rate is assumed constant and not dependent on the sample matrix. The SeH/Se rate was measured to be approximately 0.03. At $m/z = 80$ and 82 substantial interference levels were observed in real serum samples. The source for this interference is most likely BrH^+, even though the normal bromine concentration in serum is low, in the 3.5–5.5 μg L^{-1} range [32]. A BrH^+ formation rate (BrH^+/Br^+) of 5% was found when analyzing 0.1 and 1.0 mg L^{-1} bromine standards; the effect of the use of H_2 as a reaction gas on this formation rate was not investigated, but is likely increased as the SeH formation increases. The sulfur concentration in serum is relatively high, around 1,300 mg L^{-1} [32], but no SO_3^+ ions were observed on $m/z = 80$ or 82 analyzing sulfate standard solutions up to a concentration of 100 mg L^{-1}; hence, apparently the SO_3^+ ions are removed by reaction with the H_2 gas in the octopole reaction cell. Interferences from Kr^+ ions on $m/z = 80$ and 82 are eliminated via blank subtraction as the main source of Kr^+ ions is the argon gas and therefore the Kr^+ signal can be assumed to be constant. Based on these observations it was decided not to use the ^{80}Se and ^{82}Se isotopes for quantitative analyses.

With a H_2 reaction gas flow in the 2.6–3.4 mL min^{-1} range, typical background values were ^{76}Se and $^{78}Se77Se$ and $^{82}Se80Se76Se$, ^{77}Se, ^{78}Se, and ^{82}Se, whereas the Ar_2^+ interference on $^{80}Se^+$ is not fully removed at these reaction gas flows. The conclusion drawn from the interference study was that ^{76}Se, ^{77}Se, and ^{78}Se should be used for quantitative analyses. No significant difference was found in the total concentrations determined with these isotopes, and the average of the three values was used as the final result. Monitoring the concentrations calculated using ^{76}Se, ^{77}Se, and ^{78}Se serves as an extra interference control. Any significant difference between concentrations obtained for the three isotopes would indicate the presence of unresolved spectral interferences.

Optimization of H_2 reaction gas flow

Proper optimization of the H_2 gas flow is important to facilitate sufficient removal of polyatomic interferences without decreasing the analyte signal significantly. The normal procedure involves analysis of an analyte standard and a blank solution at varying H_2 flows, the signal-to-background ratio (SBR) is then calculated, and the H_2 flow corresponding to the highest SBR is chosen as the optimum flow. This approach is problematic since it involves determining very low background levels (< 5 cps), where the largest noise source is the signal itself and not the background level; this make it very difficult to determine the actual background level and hence the precise SBR and H_2 flow. Our goal is to develop a simple optimization procedure that does not include the measurement of background levels and is based on either the signal-to-noise levels or changes in polyatomic/background ion formation patterns with changing H_2 flow.

Optimization using polyatomic background ions

The pressurization of a collision/reaction cell with a reaction gas is believed to change the general ion chemistry and hence also potentially the formation or reduction of background and polyatomic ions. This was used by Ingle et al. [33] to optimize the H_2 flow using the ratios between background ions, e.g., the m/z $21(H_3^{18}O^+)/31(NOH^+)$ ratio, by applying a Thermo Elemental VG PQ ExCell ICPMS instrument with a hexapole reaction cell pressurized with a mixture of He and H_2. The optimum of the "21/31" ratio was shown to correspond to the optimum signal-to-noise ratio (SNR) for ^{80}Se. The change in formation rate of polyatomic ions was believed to be driven primarily by the presence of water vapor in the cell and changes in chemical reactions with water induced by changes in H_2 gas flow [33]. Inspired by that work we investigated the change in formation rate of several polyatomic and background ions, e.g., $m/z = 19(H_3^{16}O^+)$, $21(H_3^{18}O^+)$, $31(NOH^+)$, $36(Ar^+)$, $37(H(H_2O)_2^+)$, and $55(H(H_2O)_3^+)$; all ions were found to decrease at approximately the same rate with increasing H_2 gas flow. The most likely explanation for this is that with increasing H_2 flow the number of collisions between H_2 and background ions increases leaving the larger background and polyatomic ions with a kinetic energy too low to jump the potential barrier between the octopole and the quadrupole analyzer (≈1–2 V). Consequently, no patterns useful for optimization purposes were found for any ions or ion ratios. These findings do not agree with Ingle et al. [33], but are in agreement with a study by Yamada et al. [34], who investigated a similar octopole reaction cell system and found no evidence that the water vapor or water-related ions reacted with H_2 in the cell or had any effect on the removal of Ar^+, ArO^+, and Ar_2^+.

Optimization using the signal-to-noise ratio

A function for the total noise on the $m/z = 76$, 77, and 78 signals (selenium plus background signal) was estimated using the procedure outlined by van Veen et al. [35] in which the signal %RSD is related to α and β, where α is the flicker noise coefficient and β is the signal noise induced by counting statistics (see Table 3). The parame-

Table 3 Estimation of a noise function for the ^{76}Se, ^{77}Se, ^{78}Se signals

$$\mathrm{RSD}(x) = \sqrt{\alpha^2 + \frac{\beta}{x}}$$
yields
$$\mathrm{RSD}(x) = \sqrt{0.76^2 + \frac{570}{x}} \quad \text{or} \quad s = \frac{x}{100} \times \sqrt{0.76^2 + \frac{570}{x}}$$

ters α and β were estimated from the %RSD values calculated from 50 repeated measurements of the actual signal (counts in 0.1 s) on $m/z = 76$, 77, and 78 in blank and standard solutions (0, 3, and 5 μg L^{-1}). Values of 0.76 and 570 were found for α and β, respectively. The noise function and the estimated SNR function are drawn in Fig. 1. An α value of 0.76 indicates that the %RSD approaches 0.76% for high ion counts, which is in good accordance with experimentally obtained values. From the figure it can also be concluded that the noise on the selenium signals obtained for the serum sample analysis, which contained 2–3 μg L^{-1} after dilution (60–150 counts/0.1 s), is still limited by counting statistics, but well above the detection limit. Figure 2 shows the relative signal and the SNR for ^{77}Se, ^{78}Se, and ^{80}Se determined in a 10 μg L^{-1} standard solution as a function of H_2 gas flow. Several interesting features are apparent:

1. ^{80}Se behaves very differently to ^{77}Se and ^{78}Se, since the Ar_2^+ interference on ^{80}Se is much larger than the Ar_2^+ on ^{78}Se and not readily removed. It was concluded that ^{80}Se should not be used for a general selenium optimization as a significant higher H_2 flow (causing a unnecessary loss of sensitivity) is needed to eliminate the interference on this isotope compared with the other less severely interfered selenium isotopes (e.g., ^{76}Se, ^{77}Se, and ^{78}Se).

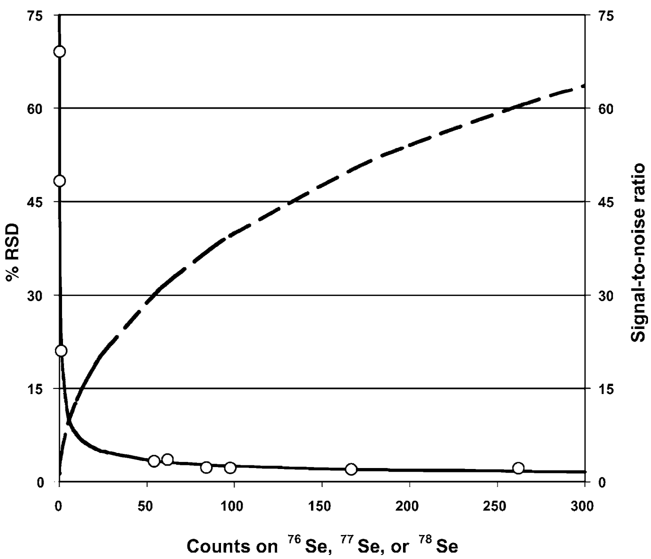

Fig. 1 Measured uncertainty for the selenium signals on $m/z = 76$, 77, and 78 (*open circles*), the estimated noise function (*solid curve*), and the estimated signal-to-noise ratio function (*dashed curve*)

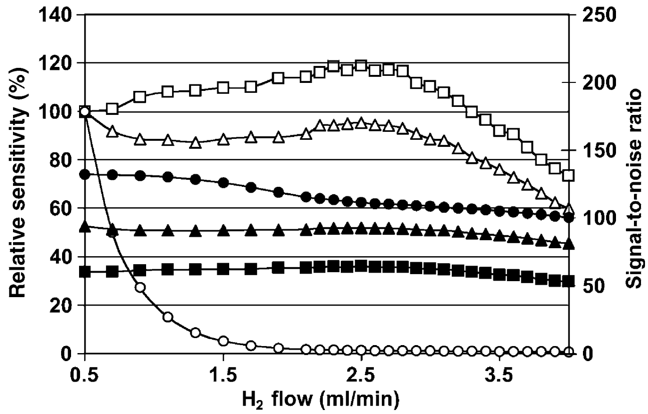

Fig. 2 Relative signal sensitivity (*open labels*) and SNR (*filled labels*) as a function of H_2 gas flow rate in the octopole reaction cell for ^{77}Se (*squares*), ^{78}Se (*triangles*), and ^{80}Se (*circles*)

2. ^{77}Se (practically un-interfered) and ^{78}Se behave very similar (i.e., the Ar_2^+ on ^{78}Se is removed almost completely at a relatively low H_2 flow, 1.5 mL min^{-1}). They both have maximum sensitivity at approximately 2.7 mL min^{-1}, a maximum sensitivity caused by collisional focusing. A similar behavior was found for ^{76}Se.
3. The SNR for ^{77}Se and ^{78}Se are less sensitive to change in H_2 gas flow than expected.

From these findings we decided to use the SNR of ^{77}Se and ^{78}Se for optimization of the H_2 gas flow, the optimum flow being the point just before the SNR starts to drop, i.e., lowest possible background whilst still maintaining a good SNR, which coincide with the point just before the signal sensitivity starts to drop significantly. The loss of sensitivity at high H_2 gas flows is due to kinetic energy discrimination or scattering. In Fig. 2 this is at a H_2 gas flow of around 2.9 mL min^{-1}. This optimization was performed daily by recording the signal sensitivity at different H_2 flow rates for ^{77}Se and ^{78}Se in a 10 μg L^{-1} selenium standard. Compared to the traditionally recommended SBR optimization procedure, the optimum H_2 flow found with the above signal-to-noise method is typically 0.3–0.5 mL min^{-1} lower thus resulting in a higher selenium sensitivity during analysis. In addition the developed method is less time-consuming and more precise, as it does not involve the evaluation of a series of measurements of a low background level.

Quality control

The quality control for this analytical method was performed at two levels, within-laboratory quality control and external quality control. The within-laboratory quality control consists of the regular quality control tests carried out for routine analysis, e.g., background measurement, duplicate analysis of real samples, and measurement of a certified reference material, whereas the external quality control is duplicate analysis of real serum samples in a pattern designed by the NCI unknown to the analyst at the time of analysis.

Within-laboratory quality control

The detection limit was calculated as three times the standard deviation on the measurement of 24 blank solutions (0.16 M HNO_3) over 3 days. The detection limits were 0.10, 0.13, and 0.10 μg L^{-1} for ^{76}Se, ^{77}Se, and ^{78}Se, respectively. The average serum selenium concentration was 137 μg L^{-1}, corresponding to a concentration of 2.7 μg L^{-1} after dilution, approximately 25 times higher than the detection limit.

The short-term or within-batch repeatability (a batch equals ten serum samples) of the method was controlled by measuring one in every ten serum samples twice. The goal was that no deviation between duplicates was to exceed 20% CV. The results for the analysis of 182 duplicate analyses are shown in Fig. 3; the average deviation was 6.3% and no deviation between duplicates exceeded 20%. The intra-class correlation coefficient (ICC) for the 182 duplicates was 0.97, showing that the majority of the variation in the complete dataset, as expected, was between measurements and not within duplicate measurements.

The running accuracy of the method was controlled by using a serum material certified to contain 136 ± 9 μg L^{-1} of selenium (Seronorm Serum level II, Sero A/S, Norway). On each day five batches of serum samples and six replicates of the certified reference material were analyzed. In Fig. 4 the control chart for the reference material is shown; each point represents the average of the six replicates analyzed during one day on 36 different days. The overall measured average is 145 ± 3 μg L^{-1}, which is about 6% higher than the certified value, but within the uncertainty of the reference material. The control chart illustrates that the method reproducibility is fairly good, although there are a few low values.

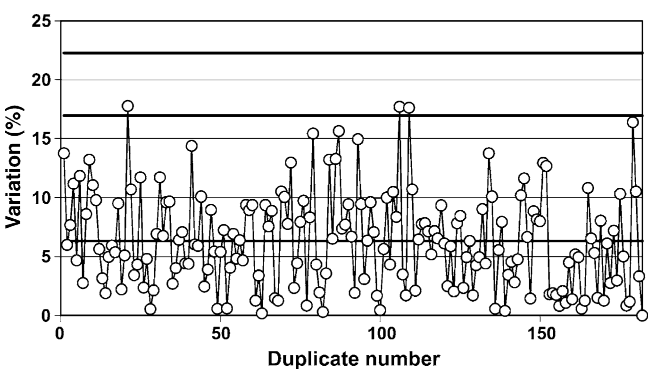

Fig. 3 Replicate analysis of serum samples

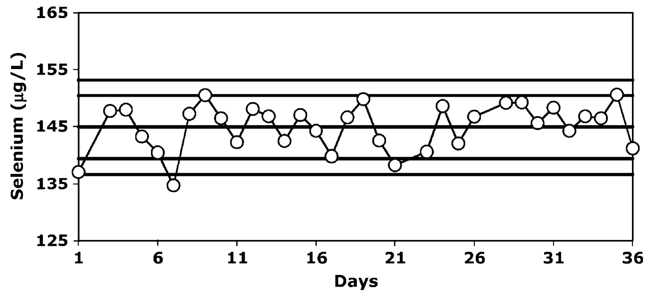

Fig. 4 Control chart for the analysis of Seronorm Serum, Trace Elements, Level II. Certified to 136 ± 9 μg L^{-1}

External quality control

The external quality control (QC) was designed to allow estimation of within-batch and between-batch variations. For QC purposes serum samples were obtained from two subjects. Two QC samples from the same subject were randomly placed in each batch alternating the two QC subjects from batch to batch. A total of 184 samples of QC subject 1 and 177 samples of QC subject 2 were analyzed. The laboratory personnel were unaware of the sample status by using the same volume and same numbering system for all quality control sample and real samples. The within-batch CV for the two QC subjects was 5.6% and 5.4%, and very similar to the values obtained for the 182 duplicate analysis described above. The between-batch CVs were 7.5% and 7.3% for the two QC subjects, which as expected are higher than the within-batch repeatability, but still acceptable.

Analysis of real samples

Table 4 shows a summary of the results of the analysis of the 768 serum samples from the control group. The average concentration is 137.1 μg L^{-1} and the majority (80%) of the samples had selenium levels in the 109.9–169.5 μg L^{-1} range. These findings are in good agreement with other US-based studies, while the concentrations found are higher than serum selenium concentrations found in Europe and New Zealand [36], where the selenium intake is lower mainly due to a generally lower selenium soil content.

Table 4 Serum selenium levels in control participants ($n = 768$)

Statistic	Selenium (μg L^{-1})
Mean	137.1
Median	133.4
Standard deviation	19.1
Minimum	73.4
Maximum	305.5
10%ile	109.9
90%ile	169.5

Measurement of zinc in serum

Zinc may also play a role in the etiology of cancer, particularly prostate cancer [37], and epidemiological studies can investigate if serum zinc is associated with cancer risk. Because the amount of stored blood specimens in epidemiological studies is limited, particularly in prospective cohort studies in which blood samples are collected from tens of thousands participants before cancer develops and are stored over many years, methods that can estimate selenium and zinc in the same sample are very attractive. As a small pilot project a subset of the serum samples ($n=200$) was analyzed for zinc. The aim of the pilot study was to test if the method developed for selenium could be used for zinc analysis simply by measuring the $m/z=66$, 67, and 68 masses without doing any further optimization or taking any additional measures of interference removal. The only change was that samples for the addition calibration were now spiked with a mixed standard solution of selenium and zinc (1:10 ratio). In order to keep the method simple the zinc isotopes were measured with H_2 gas in the octopole cell, although a higher sensitivity can be obtained in the standard mode without a reaction/collision gas. For the Seronorm Serum level II reference material, which is certified to 920 ± 60 μg L^{-1}, a value of 911 ± 75 μg L^{-1} ($n=31$) was obtained. The results were calculated as the average of the three zinc isotopes as no significant differences between the results for the three zinc isotopes were found. The detection limit (3 s) for all three zinc isotopes was around 0.2 μg L^{-1}, well below the average zinc concentration in the sample solution of approximately 20 μg L^{-1} after the 50-fold dilution. For the 200 serum samples analyzed, the average was 1,134 μg L^{-1}, the median 1,011 μg L^{-1}, and the range was 640–2,725 μg L^{-1}, which is in reasonable agreement with the normal range of 600–1,200 μg L^{-1} provide by Caroli et al. [32] for zinc in human serum. The conclusion of the pilot study was that the method developed for selenium analysis indeed can be used also for zinc analysis: this is an important result as the same sample can be used to measure both selenium and zinc, an efficient use of the precious serum samples.

Conclusion

A simple and robust routine method for the determination of serum selenium has been developed. Interferences from Ar_2^+ and $ArCl^+$ were successfully eliminated in an octopole reaction/collision cell pressurized with H_2. The method has low detection limits (0.1 μg L^{-1}), good precision and accuracy, and a high sample throughput (i.e., 50–100 samples on a daily basis), making this method ideal for the determination of serum samples from an epidemiological study, where analysis of a high number of samples, often $>1,000$, is required. In addition a novel simple H_2 flow optimization procedure are suggested which allows a fast and accurate optimization.

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